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Ken Mitton uses ChIP-on-chip to study gene expression in the retina

CBR member **Ken Mitton**, of the **Eye Research Institute**, is using state-of-the-art **biotechnology** to better understand what genes are active during the development of the eye. His team's most recent results were published in the journal **Molecular Vision** (Volume 16, Pages 252-271): "**Temporal ChIP-on-Chip of RNA-Polymerase-II to detect novel gene activation events during photoreceptor maturation**" Mitton studies gene expression in the neural **retina** of mice, but many of the same genes are found in humans, and could have important implications for retinal disease.

"**ChIP-on-chip**" is the method Mitton uses to map gene expression. The first "ChIP" stands for **Chromatin Immunoprecipitation**. The basic idea is that in order for a **gene** to be activated (so it produces an **RNA** molecule, a process called **transcription**, which is the first step in making a **protein**), the enzyme **RNA polymerase II** must bind to the **DNA**. During ChIP, the RNA polymerase II is **cross-linked** to the DNA (forming a strong bond), the DNA is broken into small pieces, and then an **antibody** (the key molecule of the **immune system**) is used to selectively **precipitate** out of solution the DNA that is bound to RNA polymerase II. The DNA in the precipitate can be isolated and then the antibody removed. In this clever way, only those regions of DNA containing active genes are separated from all the rest of the DNA.

The second "chip" refers to a DNA **microarray**. Mitton's group used a particular DNA-Chip array from **Affymetrix**, which is made using photosynthetic manufacturing processes derived from computer chip industry. Over 5-million short DNA sequences, 25 bases long, are made in an area the size of a dime. These short sequences correspond to short segments of the DNA sequence in the control (switching) regions of 26,000 mouse genes. Mice and humans essentially have the same set of 26,000 genes, distributed over 3-billion base pairs of DNA. DNA is double-stranded, so these 5-million single stranded DNA spots can bind the single stranded DNA captured by the Chromatin immunoprecipitation. The captured DNA is tagged with fluorescent molecules, and once bound to spots on the Chip-array they are detected by a laser. This type of array is only possible because we have the complete sequence of the mouse genome; any DNA sequence location is now known. In the case of RNA-Polymerase-II ChIP-on-Chip, the laser detects spots that identify and map where RNA-Polymerase-II was caught while transcribing the activated gene. The advantage of this microarray technology is that all the spots are analyzed at the same time, as opposed to having to do a separate analysis for each spot individually. This kind of experiment is also called genome-wide analysis, and it is also possible with human tissues using other Chip-arrays representing human genome sequence. This method detects many gene activation events that are not seen by methods that look for changes in the levels of messenger RNA (mRNA).

In mice, and other animals born with closed eyes, the retina is still forming after birth. By comparing retinas before and after maturation of photoreceptor cells, Mitton's experiment determined which genes turn on as the retina becomes functional for vision. Over 800 novel gene activations were discovered that were not detected by previous analysis of mRNA alone. Many OU undergraduate students are benefiting from advanced training in this project, using this results to find candidate disease genes using bioinformatics tools (Jason Sotzen, Megan Stewart, Dan St. Aubin, Wojciech Gryc, and Allyson Engle). This part of the work involves examining the 800 mouse genes and finding their human counterparts (homologous genes). Again, because of the human genome project, OU students could then find the locations of the human genes. Next, the determine which of these human genes fall within the mapped intervals of 50 inherited retinal diseases that have not yet been identified to a specific gene. Of the 800 genes examined in this way, 140 fall within the mapped intervals of unidentified retinal dystrophies. The lab is already confirming that many of these genes are specifically expressed in photoreceptor neurons of the retina. The paper concludes that

"We have analyzed the activation of thousands of genes during terminal maturation of a neural tissue using ChIP-on-chip to map Pol-II [RNA polymerase II] association around TSSs [Transcript Start Sites]. Relative increases in Pol-II binding could accurately predict genes activated during maturation of **photoreceptors** in the retina. Pol-II ChIP-on-chip detected the activation of several hundred genes in addition to those known from previous expression microarrays analysis. These represent a substantial and novel source of candidate genes that support retinal function. Many of the genes also have human **homologs** located within the mapped intervals of currently unidentified human retinal diseases, which we continue to explore."

First author on the paper is Padmaja Tummala, formerly a post doctoral fellow in Mitton's lab. Also contributing were other members of Mitton's team, including Raghuvier Mali (post doc), Xiao Zhang (laboratory technologist), and Ed Guzman (research technician). The research was supported in part by a grant from the **National Eye Institute**, one of the **National Institutes of Health**.

SUMMARY

Ken Mitton and his team or Eye Research Institute investigators uses biotechnology to determine which gene as active in the developing retina.

Created by Brad Roth (roth@oakland.edu) on Tuesday, April 6, 2010

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